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A simple electroanalytical procedure for the determination of calcium in biodiesel

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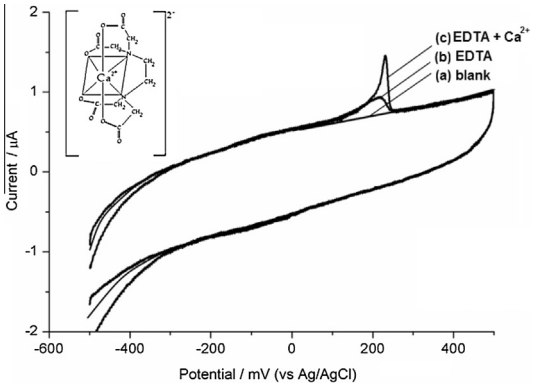
HIGHLIGHTS

- Alternative analytical procedure for the determination of calcium in biodiesel.
- Quality of biodiesel.
- Interference of magnesium in the determination of calcium.
- Calcium–EDTA complex.

GRAPHICAL ABSTRACT

Voltammograms showing the qualitative reactivity for the Ca–EDTA system in pH 9.4 (ammonium buffer 0.01 mol L^{-1}) on the glassy carbon electrode surface (υ = 100 mV/s; (a) bare electrode; (b) 4×10^{-3} mol L^{-1}EDTA on the CGE; (c) = (b) after addition of 1×10^{-4} mol L^{-1} Ca^{2+}.



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ABSTRACT

An alternative method is proposed for the determination of calcium in biodiesel samples using square-wave voltammetry and a glassy carbon electrode in a solution containing EDTA. A microwave assisted acid digestion of the biodiesel samples was carried out before analysis. Experimental parameters such as deposition potential, deposition time, frequency, amplitude, step potential, were optimized for the purpose of determination of trace calcium ion in 1×10^4 µmol L^{-1} ammonium buffer solution (pH 9.4) in the presence of 400 µmol L^{-1} of EDTA. Under optimal conditions, the limit of detection was 1.6×10^{-3} µmol L^{-1} for Ca^{2+} with a 2 min preconcentration time. In addition, the EDTA/GCE displayed good reproducibility (CV maximum of 0.70%) and accuracy (recovery around 102%) making it suitable for the determination of Ca^{2+} in real biodiesel sample.

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1. Introduction

The identification and quantification of contaminants is crucial in guaranteeing fuel quality, thus, sensitive, selective, and easy to apply analytical methods, are required. The requirements and test methods for biodiesel are usually based on the European Committee for Standardization through the norm EN 14214 [1] and the American Society for Testing and Materials (ASTM) [2] through the norm ASTM D-6751. In Brazil the Resolution number 14/2012 of the National Agency of Petroleum, Natural Gas and Biofuels (ANP) [3] supplies the specifications of pure biodiesel (B100) and the methods for its characterization.

Despite their lower concentrations, inorganic contaminants (e.g. metals) of liquid biofuels have a crucial effect on the quality of the final product [4,5]. Even small concentrations of metal contaminants showed nearly same influence on oxidation stability of Biodiesel as large amounts [6,7]. Some elements are introduced during production process (Na, K, Ca, and Mg), while other elements present in the feedstock (P, S, and Zn), or used as additives (Si, Mn, Cr, Fe, and Ni), or still during storage or transportation and require monitoring [6,7,9]. Different transition metals (iron, nickel, manganese, cobalt, and copper) are commonly found in barrels and metallurgy of storage tanks of Biodiesel [6–9]. All of these contaminants have their contents regulated by technical standards. One of the critical parameters indicated of quality control for the commercialization of biodiesel is the presence of metals [10].

Metals such as Na^+, K^+, Ca^{2+} and Mg^{2+}, alongside the non-metal P, comprise the group of elements that are quantified in accordance to biodiesel quality standards. Na^+ and K^+ originate from the biodiesel processing that uses alkaline hydroxides as reaction catalysts, Mg^{2+} and Ca^{2+} are introduced into biodiesel during the purification process as anhydrous $MgSO_4$ and CaO are used as drying agents. These chemical species may also be inherently present in the raw materials and remain in the final product [11]. The presence of such alkaline and alkaline earth ions may produce residual deposits that, for instance, clogs, the fuel injection system [10,12].

Besides calcium is present in Biodiesel it is an official parameter of the specifications of this biofuel [pure biodiesel (B100)], determined through official norms such as EN 14214 (Europe), or ASTM D-6751 (United States) and the Resolution ANP number 14/2012 (in Brazil). In agreement with these legislations, the official method for the determination of calcium is regulated by EN 14538 [1] that indicates analysis through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) where the maximum limit allowed, for the total of calcium plus magnesium cations, is 5 mg kg^{-1}. The monitoring of the concentrations of Ca^{2+} and Mg^{2+} in biodiesel is necessary due to the fact that salts of these metals form solid deposits in engines [13,14].

The determination of metallic species in fuels is often difficult and requires special care in sample preparation due to the high complexity of these matrices [14]. In the case of biodiesel, the sample preparation procedure recommended by Brazilian and by International official procedures is the dissolution of the sample in xylene and subsequent determination by either flame atomic absorption spectrometry (FASS) or inductively coupled plasma optical emission spectrometry (ICPOES) [15]. These procedures usually requires the use of organometallic standards, which are costly and unstable in solutions, and will might beprone to transport interferences if proper matrix matching between samples and standards are not made. The contaminants Ca^{2+}, Mg^{2+}, Na^+ and K^+ are the only metals that have specified allowable maximum concentrations in biodiesel. In Brazil, two official standard methods are recommended for the determination of Ca^{2+} and Mg^{2+}, which are based on the international standards: the ABNT NBR-15553, that employs ICPOES [15,16] and the ABNT NBR-15556, that employs FAAS [17].

The use of electroanalytical methods for the determination of inorganic contaminants in biofuels and liquid fossil fuels has been a subject of great interest in the last decade [18]. The application of electroanalysis for on-site measurements in the quality control of biofuels, provides remarkable advantages over spectrometric procedures [18–20], mainly due to the compact instrumentation, low-power requirements, low cost of equipment acquisition and maintenance, lower demand for samples and reagents, fast response, high sensitivity and also because of safety issues as flammable gases, that are not required in the laboratory [3].

The determination of alkaline earth metals by electrochemical techniques has generally been conducted after combining the analytes with complexing agents to enable selective and sensitive determinations. For instance, Zheng et al. used an ionic liquid (1-penthyl-3-methyl-imidazolehexafluorphosphate) mixed in a carbon paste to determine Ca^{2+}. This modified electrode enabled sensitive and fast differential pulse voltammetric responses between the concentration range from the limit of quantification (8.0×10^{-7} mol L^{-1}) to 1.0×10^{-4} mol L^{-1} [21]. Wu et al. proposed a new and simple method for the determination of Mg^{2+} and Ca^{2+} in biological samples by potentiometry. The approach relied on the reaction of either Mg^{2+} or Ca^{2+} with the complex Zn–EGTA. The determinations were made indirectly by measuring the released Zn^{2+} into the solution. These determinations were carried out in ammonium buffer at the pH 9.5 for calcium and at pH 10.2 for magnesium and the quantitative analysis was performed by the standard addition method with LOD of 4 μg L^{-1} for calcium [22]. Zezza et al. determined Mg^{2+} in biodiesel using square-wave adsorptive voltammetry and differential pulse adsorptive voltammetry using a carbon paste electrode modified with mercury. Measurements were based on the formation of complexes with sodium thiopentone at pH 10.7. The LOD value for Mg^{2+} was in the order of 10^{-7} mol L^{-1} for using of the approaches [23]. Wang et al. quantified Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} by anodic stripping voltammetry (ASV) employing a static mercury drop electrode and solochrome violet as complexing agent. In this study a sensitive way to measure metals at trace levels through adsorptive stripping method provided LOD of 26 ng L^{-1} (6.5×10^{-10} mol L^{-1}) for Ca^{2+} [24].

The successful application of electroanalytical methods in fuel and biofuels have been attributed mainly to the different strategies developed [19,20] in function of analytes and sample matrices. On the other hand special attention has been given to electrodes, detection techniques, and sample preparation protocols (when required) [18].

In this work, a very simple procedure is proposed for the electrochemical determination of calcium in order to establish an alternative method for the analysis of the quality of biodiesel samples using square-wave anodic stripping voltammetry (SWASV). The method relies on the electroactive response of the reaction product of Ca^{2+} with ethylenediaminetetraacetic acid (EDTA) at a specific pH range.

2. Materials and methods

2.1. Instrumentation

The electrochemical measurements were performed on a CV-50W potentiostat voltammetric analyzer from Bioanalytical Systems (USA) coupled to a personal computer to record the data. A glassy carbon electrode (diameter about 3.0 mm) was used with the Ag/AgCl (KCL 3 mol L^{-1}) reference electrode and a platinum wire as the auxiliary electrode. At this stage of the experiment

the glassy carbon electrode was polished with alumina 1 μm in each new experiment (new solution or new sample in the cell) to complete the removal of deposits formed and/or adsorbed in order to renew the electrode surface. The square-wave technique was used for the voltammetric measurements. The decomposition of the wet samples (in closed vessels) was made on an Anton Paar (USA) Multiwave 3000 (model XQ804) microwave oven.

2.2. Reagents and solutions

All reagents were of analytical grade. The ultrapure water (resistivity of 18.2 MΩ cm) was obtained from a Nanopure Master System (Gehaka, USA). A stock solution of Ca^{2+} 100 μmol L^{-1} was prepared from the dilution of a 1000 mg L^{-1} standard solution (Merck, Germany). The 1×10^4 μmol L^{-1} ammonium buffer solution (pH 9.4) was prepared by using NH_4Cl (Reagen, Brazil) and NH_4OH (Merck, Germany). The pH adjustments were made with NaOH (Merck) in the form of aqueous solutions. An aqueous 1×10^{-4} μmol L^{-1} EDTA solution was prepared by the dissolution of the solid EDTA (Synth, Brazil) into water. Ultrapure nitric acid (Merck) and high purity hydrogen peroxide (Isofar, Brazil) were used for sample treatment. Two biodiesel samples from the Brazilian National Petroleum Agency interlaboratory program were used to validate the method.

2.3. Sample preparation

For sample treatment, a microwave assisted digestion in a closed system was performed. The samples were prepared directly in 15 mL quartz tubes by adding 300 mg of biodiesel, 4 mL of ultrapure nitric acid, 2 mL of high purity hydrogen peroxide and 1 mL of deionized water. The vials were then properly sealed and heated in three steps described as power (W)/Ramp (W min^{-1})/Time (min): step (1) 300/10/10; step (2) 450/5/5; step (3) 0/0/20. At the end of the processing, the samples were stored under refrigeration in 15 mL polypropylene tubes. The procedure for sample preparation used was adapted from the microwave oven equipment manual and literature [25] used in this experiments to digest the samples. The quantities of sample, reagents and heating ramp were optimized by the authors themselves.

2.4. Analysis procedure

The determination of Ca^{2+} in biodiesel was performed using an electrochemical cell containing 8 mL of a 1×10^4 μmol L^{-1} ammonium buffer pH 9.4 and 400 μL of an EDTA solution 1×10^4 - μmol L^{-1}. After sample digestion the pH of the supporting electrolyte (ammonium buffer 1×10^4 μmol L^{-1}) or sample was adjusted with addition of 1 mol L^{-1} NaOH, to a value around 9.0 and then an aliquot of the sample was added into the cell. The glassy carbon electrode was polished with aluminum oxide, (1 μm grain size) at the beginning of each working day. Successive anodic scans (from −600 to 600 mV) were conducted for signal stabilization of the ligand, since it signal appears at the same potential of the Ca^{2+}–EDTA complex. Following stabilization, 2 mL of digested biodiesel were added into the cell. Sample was analyzed by SWASV using the parameters indicated in Table 1. It was performed suitable additions of micro volumes of a standard solution of a 100 μmol L^{-1} Ca^{2+} standard.

Cyclic voltammetric measurements were carried out in a −1000 to 1000 mV potential range. 10 mL of 1×10^4 μmol L^{-1} ammonium buffer solution pH 9.4 was used as supporting electrolyte. An aliquot of 400 μL of 1×10^5 μmol L^{-1} EDTA was added into the sample solution containing Ca^{2+}.

Table 1
Optimized electrochemical parameters for the determination of Ca^{2+} by SWASV.

Parameter	Specifications
Preconcentration time	120 s
Equilibration time	15 s
Initial potential	−1000 mV
End potential	+600 m V
Deposition Potential	−1000 mV
Frequency	30 Hz
Pulse amplitude	100 mV
Step potential	15 mV
Scan direction	Anodic
Scan mode	SWV

3. Results and discussion

3.1. Preliminary studies

A preliminary voltammetric study was performed to qualitatively verify the electroanalytical interaction between Ca^{2+} and EDTA, which is a classical analytical reaction often used in complexometric titrations [26]. EDTA is one of the most widely used chelating agents with six complexing groups (two nitrogen and four carboxylic groups). Data from the literature [27] and from our experiments referent to the reaction between Ca^{2+} and EDTA and by assuming only one type of ternary surface complex (Me^{2+}–EDTA), indicate the following reaction:

$$Ca^{2+} + 2HNO_3 \rightarrow Ca(NO_3)_2 + 2H^+ \quad (1)$$

where Ca^{2+} ion is dissolved in its standard or sample solution in nitric acid medium. Adding an aliquot of calcium solution to EDTA solution ($Ca^{2+} + EDTA^{4-} = CaEDTA^{2-}$) a very stable chelate [CaEDTA^{-2} ($C_{10}H_{12}O_8N_2Ca$)] is formed so a reaction proceeds quantitatively from left to right, as showed by the following equation:

$$K'_f, CaEDTA^{2-} = \frac{[(CaEDTA)^{2-}]}{[Ca]^{2+}[EDTA]^{4-}} \quad (2)$$

$CaEDTA^{2-}$ has a high formation constant ($K_f = 5.0 \times 10^{10}$). EDTA is therefore used to determine the concentration of Ca^{2+} and the selectivity is obtained by controlling the pH as discussed in the following paragraph.

EDTA complexes are highly stable but the effectiveness of the reaction with a specific metallic ion depends on the solution conditions, especially the pH of the medium. The pH interfer on complexation reaction between EDTA and metals according each equilibria, considering the stability constant of each complexes and acid ionization constants of EDTA ($pK_1 = 2.0$, $pK_2 = 2.67$, $pK_3 = 6.16$ and $pK_4 = 10.26$) [28]. As it is very known, EDTA (Y^{4-}) complexes will be more efficiently and be more stable in alkaline solution. On the other hand, complexes of most divalent metals (e.g. Ca^{2+}) are stable in ammonical solution. In the present study firstly the solution was neutralized, and then the pH was adjusted with ammonium buffer solution.

3.2. Possible mechanism for the formation of the Ca^{2+}–EDTA complex on the electrode surface

Adsorption of EDTA complex is very known [29]. The interaction of EDTA with the surface of GC (glassy carbon) electrodes can be explained considering that the different functional groups containing oxygen such as carbonyls, phenols, carboxyl acids, lactones, quinones, etc. can be present on the GC surface [30].

Reactions involving EDTA are more suitable in alkaline medium, where induction period of its oxidation reactions should be

decreased with increasing NaOH concentration [31]. Studies on the adsorption of metal–EDTA [29] show that beginning at pH 9.0 Ca^{2+} and EDTA are adsorbed at a 1:1 ratio (complex is undissociated in this region) and, as the pH decreases, the adsorption ratio decreases to zero, as no Ca^{2+} is adsorbed at pH 4.0, even though the EDTA adsorbed is equivalent to the magnitude of "free" EDTA.

Based on this achievement/findings, we propose that Ca^{2+} and EDTA can be adsorbed on the carbon surface electrode before oxidation process. The description of this interaction between EDTA and Ca^{2+} ion (Eq. (2)) with the electrode surface in alkaline medium is waited to be as presented in the reactions below:

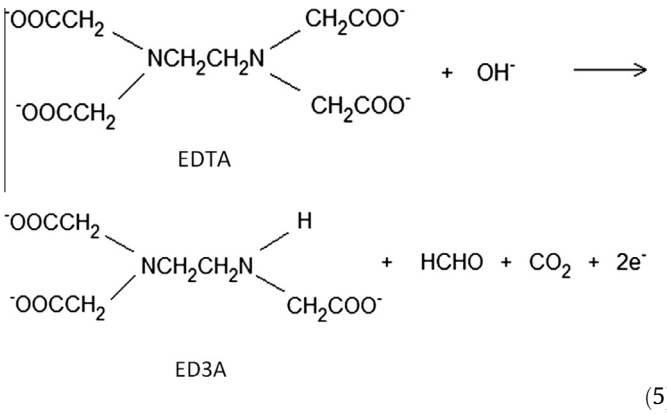
$$-COH_{(surface)} + EDTA^{4-}_{(solution)} + 2OH^{-}_{(solution)} <=> CO = EDTA^{4-}_{(adsorbed)} + H_2O + OH^{-}_{(solution)} \quad (\log\ K_{(surface)}) \tag{3}$$

where –COH represents a surface site. *K* represents the constant of this reaction. Ca^{2+} complexation on the electrode surface is presented below. Adding an aliquot of Ca^{2+} to a solution where there is an electrode with EDTA in its surface, a stable chelate of CaEDTA^{-2} ($C_{10}H_{12}O_8N_2Ca$) is waited to be formed so a reaction proceeds quantitatively from left to right as showed by the reaction below:

$$Ca^{2+}_{(solution)} + CO = EDTA^{4-}_{(adsorbed)} ==> [CO = EDTACa)^{2-}_{(adsorbed)}] \quad (E1) \tag{4}$$

In the conditions used in the present work an anodic peak current was observed around 200 mV (E_1) which was attributed to oxidation process for the complex "CO = EDTACa)$^{2-}$" accumulated onto the electrode. For this complex the redox process is atributted to EDTA ligand due the nature redox of metal calcium that present only a simple oxidation state (+2). E_1 represents the potential in which occurs the irreversible oxidation of this complex whose mechanism is discussed below.

The anodic oxidation of EDTA was studied in alkaline medium on a platinum electrode [32]. The study indicated that organic intermediates (e.g. formaldehyde) are formed during the oxidation of EDTA. Ethylenediamine also can be formed, in order to complete oxidation of EDTA to CO_2. In this study the two electrons participating in the initial EDTA oxidation reaction was determined by electrolyzing EDTA solution under constant current conditions, and measuring the CO_2 content in the alkaline solution using total inorganic carbon (TIC) analysis. Based in the literature [6] we suggest a pathway for the initial EDTA oxidation reaction related to the redox process observed in the present work:



(5)

OH– is the site where the EDTA is connected with the electrode surface, HCHO and CO^2 are the products of oxidation reaction.

Cyclic voltammetry studies were made. Firstly, for the solution containing EDTA, one scan cycle between −1000 mV and 1000 mV was applied producing irreversible oxidation peak at approximately 200 mV (Fig. 1). As the pH of the electrochemical cell was kept at 9.4, the addition of Ca^{2+} lead to the formation of the Ca–EDTA complex that produced the significant increasing of the oxidation peak at 200 mV. Such increase was proportional to the added concentration of Ca^{2+} as seen in Fig. 1. It is important to point out that cyclic voltammetry experiments made without the presence of EDTA, produced no redox peaks as Ca^{2+} was added into the cell.

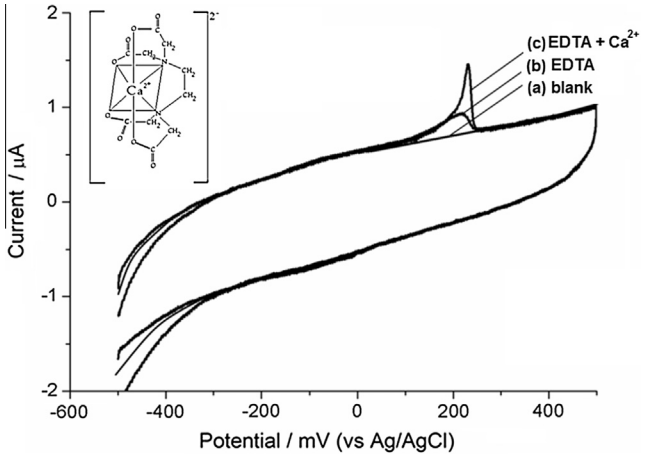


Fig. 1. Cyclic voltammetric response showing the qualitative reactivity for the Ca–EDTA system in an ammonium buffer on the glassy carbon electrode surface. Experimental conditions: supporting electrolyte: 10 mL ammonium buffer 1×10^4 - µmol L^{-1}; pH = 9.4; υ = 100 mV/s; E_i = −1000 mV; E_f = 1000 mV; (a) blank; (b) 4×10^3 µmol L^{-1} EDTA; and (c) 100 µmol L^{-1} Ca^{2+}.

3.3. Selection of experimental and instrumental conditions

In order to obtain the best conditions to perform the square-wave voltammetric determination of Ca^{2+} through the signal produced by the Ca–EDTA complex, a systematic univariate optimization was performed.

The first studied parameter was the concentration of EDTA in the electrochemical cell. In order to adjust this parameter, a compromise was made between the amount necessary to guarantee an excess of the ligand for the reaction and the generation of the lowest possible characteristic signal (low blank signal). A concen-

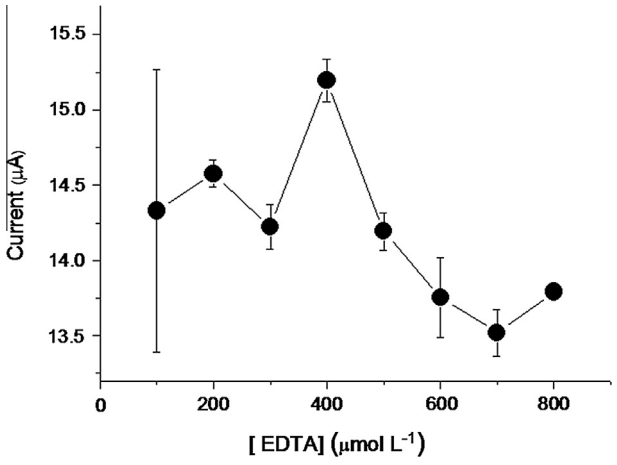


Fig. 2. Study of the effect of EDTA on Ca^{2+} concentrations, using a glassy carbon electrode in SWASVmode. Experimental conditions: supporting electrolyte: 10 mL ammonium buffer 1×10^4 µmol L^{-1}; pH = 9.4; [Ca^{2+}]: 1 µmol L^{-1}; E_{dep} = −1000 mV; t_{pre} = 120 s; amplitude = 100 mV; Freq = 30 Hz; Step E = 15 mV.

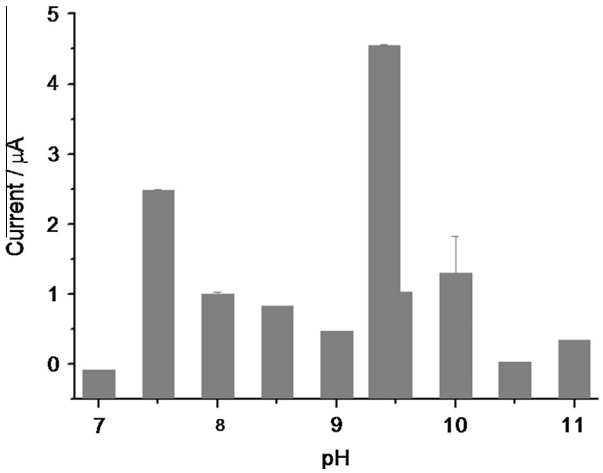


Fig. 3. Effects of pH on the peak current using a glassy carbon electrode in SWASV mode. Experimental conditions: supporting electrolyte: 10 mL ammonium buffer 1×10^4 μmol L^{-1}; [Ca^{2+}] = 1 μmol L^{-1} + EDTA; E_{dep} = −1000 mV; t_{pre} = 120 s; amplitude = 100 mV; Freq = 30 Hz; Step E = 15 mV.

tration range of EDTA between 100 μmol L^{-1} and 800 μmol L^{-1} was evaluated in a cell containing Ca^{2+} at 1 μmol L^{-1} (Fig. 2), which guaranteed a large excess of the ligand. It was observed that the maximum signal for calcium was obtained when the EDTA concentration was 400 μmol L^{-1}. The blank signal characteristic from the EDTA was low and constant over the entire studied concentration range. The pH of the supporting electrolyte (ammonium buffer 1×10^4 μmol L^{-1}) was adjusted with addition of 1×10^6 μmol L^{-1} NaOH and, as expected, the characteristic signal from the analyte was maximum at a pH 9.4 (Fig. 3) as this pH favors the formation of the Ca–EDTA complex.

According to the optimization of the other parameters of SWASV, the values chosen for E_{dep}, t_{pre}, Amp, Freq and Step E were −1000 mV, 120 s, 100 mV 15 Hz and 30 mV, respectively, because they meet the expectations in terms of resolution and sensitivity for the system and the experimental conditions in question. A detailed insight into the behavior of these parameters studied can be found in the Supplementary material (Figs. S1 and S2).

3.4. Analytical performance

The analytical parameters of the method were obtained using the optimized conditions for determination of Ca^{2+} in the presence of EDTA. A sequence of voltammograms of solutions containing an increasing concentration of Ca^{2+} is shown in (Fig. 4A), indicating the directly proportional relationship between peak height and concentration of Ca^{2+} added into the cell. The analytical curve (Fig. 4B) was modeled by the equation $I_p = 9.05 \times 10^{-6}$ - μA + 3.19 μA mol^{-1} L [Ca^{2+}] presenting a linear behavior characterized by a correlation coefficient of 0.994.

Limit of detection (LOD) [3 (SD)/slope] has been calculated through the signal of Ca^{2+} from 10 replicates of the analytical calibration for the solution of the blank (solution with all components except the sample), and discounting the sign of EDTA. The signal Ca^{2+} corresponding to the intercept at each analytical curve, and standard deviation (SD) of the mean value of these intercepts were used to calculate DL. The calculation of limit of quantification (LOQ) [10 (SD)/slope] was made from the same experiments following the same procedure. These values were only calculated after obtain stable signals for the blank. The LOD (1.6×10^{-3} - μmol L^{-1}) and LOQ values (5.3×10^{-3} μmol L^{-1}) in the cell showed a good sensitivity of the method.

A comparison of the analytical parameters of the present proposed method with other methods found in the cited literature [21,22,24,33–36], referent to the determination of calcium with EDTA and other ligands and electrodes, was held (see Table 2).

The main possible interferences in this method are related with the presence of Mg^{2+}, Ba^{2+} and Sr^{2+} due to the reactivity of EDTA with these cations. The selectivity of the method was evaluated by investigating interferences caused by the presence of Mg^{2+}, Ba^{2+} and Sr^{2+} in controlled recovery tests. The influence from these alkaline earth ions was evaluated by the ratio values between the signal obtained from a Ca^{2+} standard (1 μmol L^{-1}) and the signal obtained in a mixture of this Ca^{2+} standard with the increasing proportions of another alkaline earth cations (i Ca^{2+}/i (Ca^{2+} + interferent)). Values close to unity indicated no interference while values less than 1 indicated that there was the increasing of the measured signal due to the interference (see Table 3). The presence of Mg^{2+}, Ba^{2+} and Sr^{2+} in the same concentration level of Ca^{2+} (molar ratio of 1:1) imposed critical interferences to the method. However, this fact is not of much concern since Ba^{2+} and Sr^{2+} are not commonly found in industrial biodiesel samples. The origin of these elements should be from their use as base catalysts in the transesterification reaction that produces biodiesel [37]. However, they are not commonly used for such purposes.

Mg^{2+} is generally determined in biodiesel along with calcium, even by official methods (EN 14214, ASTM D-6751 and RANP 14/2012). As expected, the presence of Mg^{2+} can impact the Ca^{2+} determination, mainly considering the classic affinity of EDTA with these two ions. The method selectivity towards Mg^{2+} is achieved in solutions containing Ca^{2+}:Mg^{2+} molar rations of 1:0.2, which means that the magnesium concentration in the sample can reach a limit of up to one fifth of the concentration of calcium. In fact the magnesium relative content expected in biodiesel is lower than that of calcium for two reasons. Firstly, due to the fact that calcium is more employed than the magnesium as catalysts in the transesterification

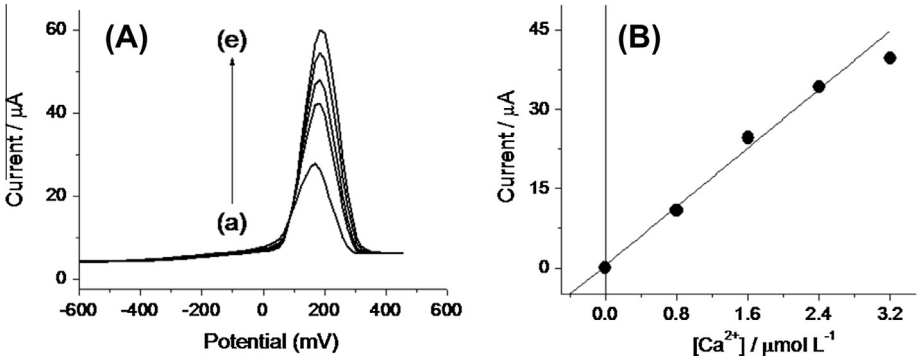


Fig. 4. Voltammograms (Fig. 4A) and analytical curves (Fig. 4B) for the supporting electrolyte solution, ammonium buffer 1×10^4 μmol L^{-1}; pH = 9.4; 400 μmol L^{-1} EDTA with additions of aliquots of Ca^{2+}standard solutions in the concentration range using SWASV and CG (glassy carbon). E_{dep} = −1000 mV; t_{pre} = 120 s; Amp. = 100 mV; Freq. = 30 Hz; Step E = 15 mV; (a) 400 μmol L^{-1} EDTA; (b) 0.8 μmol L^{-1} Ca^{2+}; (c) 1.6 μmol L^{-1} Ca^{2+}; (d) 2.4 μmol L^{-1} Ca^{2+}; and (e) 3.2 μmol L^{-1} Ca^{2+}.

Table 2
Comparison among voltammetric methods for Ca^{2+} determination.

Data	Ca^{2+}/EDTA (GCE)[a]	Ca2+/EDTA (GCE-MFE) [22]	Ca^{2+}/EDTA (H.M.D.E)/(D.M.E)/(P.G.E) [33]	Ca^{2+}/BPR (CP-MFE) [34]	Ca^{2+}/(T-CPE) e (IL-CPE) [21]	Ca^{2+}/SVRS (H.M.D.E) [24]	Ca^{2+}/(PoPD/MWNTs) (GCE) [35]	Ca^{2+}/CPE [36]
Sample	Biodiesel	Plasma and human hair	Synthetic samples	Biodiesel	Urine	–	Calcium dobesilate	Drugs
Technique	CV/SWASV	AdSV	Polarography	SWASV/DPSV	CV/DPV	AdSV	CV/SWASV	Potentiometry
Supporting electrolyte ($\mu mol\ L^{-1}$)	Ammonium buffer 1×10^4	Ammonium buffer	Ammonium buffer	Borax buffer 1.5×10^4	H_2SO_4 5×10^4	Phosphate buffer 5×10^4	H_2SO_4 1×10^5	Tetraethyl-ammonium perchlorate 1×10^5
pH	9.4	9.54	9.3	9.0	1.3	9.5	1.0–6.5	5.5
VC%	0.70	4.7	–	–	1.3	–	1.8–4.6	–
Sensibility ($\mu A\ mol^{-1}\ L$)	3.19	–	–	8.2×10^{-3}/750	4.19×10^6	5.48×10^5	–	–
LOD ($\mu mol\ L^{-1}$)	1.6×10^{-3}	0.1	–	3.5/0.039	0.4	6.5×10^{-4}/1.1×10^{-4}	0.035	0.079
LOQ ($\mu mol\ L^{-1}$)	5.3×10^{-3}	–	–	12/0.13	–	–	–	–

[a] present work.

Table 3
Influence of foreign cations on Ca^{2+} peak currents in optimized conditions.

Interferent	Proportion (Ca: interferent)	$i_{Ca}/(i_{Ca} + i_{Interferent})$	Proportion (Ca: interferent)	$i_{Ca}/(i_{Ca} + i_{Interferent})$
Mg^{2+}	1:0	1.00	1:0	1.00
	1:0.1	1.00	1:1	0.61
	1:0.2	0.95	1:2	0.48
	1:0.3	0.82	1:3	0.42
	1:0.4	0.74	1:4	0.33
Ba^{2+}	1:0	1.00	1:0	1.00
	1:0.1	1.00	1:1	1.08
	1:0.2	0.97	1:2	0.86
	1:0.3	0.44	1:3	0.71
Sr^{2+}	1:0	1.00	1:0	1.00
	1:0.1	0.50	1:1	0.55
	1:0.2	0.30	1:2	0.37
	1:0.3	0.25	1:3	0.38

process of biodiesel. Secondly, the concentration of magnesium in the water used to wash biodiesel is also expected to be smaller than that of calcium.

So considering the expected concentrations of Ca^{2+} and Mg^{2+} in biodiesel samples, the classical and waited interference of Mg^{2+} in the Ca^{2+} determination is not considered a critical problem, but otherwise, the fact of EDTA react with this ion indicate that the interference of Mg on Ca determination, observed in the present work, can be a possible alternative to the official methods to determine the total content of these two elements into biodiesel.

3.5. Application of the proposed method in real biodiesel samples

Fig. 5A shows the voltammograms of the increasing concentrations and the corresponding analytical curve (Fig. 5B) used for the determination of Ca^{2+} in the digested samples of biodiesel obtained from soybean and bovine fat. The voltammograms were performed under optimal conditions by successive additions of increasing concentrations of standard solution of Ca^{2+}.

In order to measure the current of Ca^{2+} firstly the current for the EDTA redox process was measured after the complete stabilization of signal (by repeated cyclic scanning of the potential). After addition of Ca^{2+}, an increase in the peak at the same position of EDTA was observed, whose difference in current is the result of the redox process of Ca^{2+}–EDTA complex. The difference in current was attributed to the signal characteristic referent to the Ca^{2+} in this complex.

Two biodiesel samples obtained from the interlaboratory official program of the Brazilian Government, coordinated by The Brazilian National Agency of Petroleum, Natural Gas and Biofuels (from Portuguese: Agência Nacional do Petróleo, Gás Natural e Biocombustíveis – ANP) were used in the present study. The biodiesels used (soybean and fat bovine) were provided by manufacturers approved by ANP, and were obtained via methylic route, using KOH

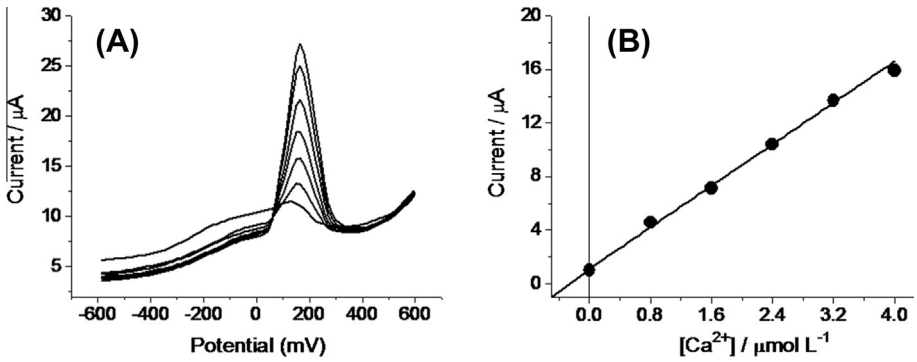


Fig. 5. Voltammograms (Fig. 5A) and analytical curves (Fig. 5B) for digested biodiesel samples with successive additions of the Ca^{2+} standard solution in a concentration range in a supporting electrolyte solution, ammonium buffer 1×10^4 $\mu mol\ L^{-1}$; pH = 9.4; 400 $\mu mol\ L^{-1}$ EDTA using SWASV and CG (glassy carbon). $E_{dep} = -1000$ mV; $t_{pre} = 120$ s; Amp. = 100 mV; Freq. = 30 Hz; Step E = 15 mV; (a) 400 $\mu mol\ L^{-1}$ EDTA; (b) sample; (c) 0.8 $\mu mol\ L^{-1}$ Ca^{2+}; (d) 1.6 $\mu mol\ L^{-1}$ Ca^{2+}; (e) 2.4 $\mu mol\ L^{-1}$ Ca^{2+}; (f) 3.2 $\mu mol\ L^{-1}$ Ca^{2+}; and (g) 4 $\mu mol\ L^{-1}$ Ca^{2+}.

Table 4
Analytical parameters obtained from the determination of Ca^{2+} in a sample of biodiesel by SWASV.

Parameter	$[Ca^{2+}]$ (μmol L^{-1}) (biodiesel of soybean/bovine fat year 2009)	$[Ca^{2+}]$ (μmol L^{-1}) (biodiesel of soybean/bovine fat year 2010)
S_1^a	2.84	0.34
S_2^b	2.82	0.338
S_3^c	2.86	0.343
$X_{average}$	2.84	0.34
SD	0.02	3×10^{-3}
CV%	0.70	0.59
μ (n = 3.95%)	2.84 ± 0.0497	0.34 ± 4.97 × 10^{-3}

$S_{(1,2,3)}$: samples of biodiesel[a,b,c].

as catalyst. These samples were used to test the method proposed for the determination of Ca^{2+}.

Microwave sample dissolution was made before the addition into the electrochemical cell. Sample and standard solution compositions were the same and all values represent the average of three replicates.

The precision of the method was evaluated through the intermediary precision measured from the analysis performed in three different days and the result of variation coefficient obtained in the Ca^{2+} determination were 0.70 and 0.59 for mixed samples from bovine fat and soybean (produced in 2009 and 2010), respectively (see Table 4). Recoveries in two concentration levels (2.5 μmol L^{-1} and 6.4 μmol L^{-1}) for spiked test samples were obtained calculating the amount recovered, by comparing the added value with the value measured by this method. Average results of recoveries for these real samples were between 102% and 103%, therefore close to 100%. According to the results, the proposed method was successfully applied for the determination of Ca^{2+} in biodiesel samples.

4. Conclusions

The method proposed in this paper enabled the sensitive determination of Ca^{2+} in biodiesel after acid digestion of the samples. Precision and accuracy were satisfactory as indicated by the results obtained in analysis of real samples. The amount of EDTA that enabled the appearance of the analyte signal was adjusted to produce low background yet guaranteeing excess of the complexing agent. The use of re-dissolution approach and square-wave pulse enabled good sensitivities (limits of detection in the cell in the 10^{-3} - μmol L^{-1} level). Interference test showed that Sr^{2+}, Mg^{2+} and Ba^{2+} would not produce interferences when their concentrations are smaller than the one of Ca^{2+} but the presence of these elements in the same concentration level of Ca^{2+} (molar ratio of 1:1) impose critical interferences to the method. The characteristic sensitivity of SWASV, the simplicity of the procedure and the use of readily available and cheap reagents makes the proposed method very attractive for biodiesel analysis.

Considering the expected concentrations of Ca^{2+} and Mg^{2+} in biodiesel samples and the affinity of EDTA for these two ions it is possible to use the method as an alternative to the official ones to determine the total content of Ca^{2+} and Mg^{2+} in biodiesel.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fuel.2013.07.088.

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